ON THE ANTIPNEUMOCOCCAL POWERS OF THE BLOOD IN PNEUMONIA.*

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Various observers—Wolf,[1] Boettscher,[2] Neufeld and Haendel,[3] and Strouse[4]—report an increase in opsonins during lobar pneumonia, and the first observer found that after a preliminary fall the opsonins increase gradually until a maximum is reached shortly after the crisis. It is not possible to demonstrate this rise except with avirulent organisms, and as a result some doubt has been expressed as to whether this is really of importance in combating infection with the virulent and, at least in artificial conditions, non-phagocytable pneumococci such as are isolated from actual cases of pneumonia.

Some investigators, indeed, have been unable to demonstrate any existence of protective antibodies of any sort in lobar pneumonia —among them Washbourn,[5] and Seligmann and Klopstock.[6] Their methods, however, were unsuited to show the presence of opsonins, and the fact that in their hands pneumonic serum failed to protect laboratory animals has apparently been satisfactorily explained by Neufeld and Haendel.[7]

The following work was undertaken to determine whether or not in cases of lobar pneumonia an increase of antibodies, and more especially of opsonins, could be demonstrated by the plate method. It was intended that this should be part of a more extended study; but as circumstances have arisen that make it impossible to continue the work at this time, the results so far obtained are presented here, although of a very fragmentary character.

The methods of investigation were as follows:

Pneumonic serum.—Specimens of serum, obtained with aseptic precautions, were separated from the clot and preserved in sealed tubes in the ice-box until the entire series from a given case of

* Received for publication November 20, 1911.

[1] *Jour. Infect. Dis.*, 1906, 3, p. 731.

[2] *Deut. Arch. f. klin. Med.*, 1910, 98, p. 93.

[3] *Arch. a. d. k. Gsndhtsamte*, 1910, 34, p. 166.

[4] *Jour. Exper. Med.*, 1911, 14, p. 109.

[5] *Jour. Path. and Bacteriol.*, 1895, 3, p. 214.

[6] *Ztschr. f. Immunitätsf.*, 1910, 4, p. 103.

[7] *Loc. cit.*

pneumonia had been obtained. Mixtures were then made up of equal quantities of serum, of a suspension of fresh, washed, normal human leukocytes, and of a very dilute suspension of a 24-hour growth of pneumococci on blood agar, a control mixture being made up with normal human serum. Immediately after mixing, equal small quantities measured by means of a capillary pipette were plated out on blood agar. The mixtures were then incubated three to four hours, and at the end of this time similar measured quantities were again plated out. After 18 to 24 hours' incubation, the number of colonies on each plate was counted.

Pneumonic leukocytes.—Specimens of serum were collected and treated as before. In addition, sterile blood was run into sodium citrate solution, and the corpuscles obtained in this way were washed immediately, along with a corresponding suspension of normal human corpuscles. The corpuscles were then stored in the ice-box over night; on the following morning they were separated from the supernatant salt solution by centrifugation, and counts were made both of the patients' and the normal leukocytes. One of the two suspensions was then diluted with its corresponding suspension of red blood cells, from which the leukocytic "cream" had been removed, to a degree sufficient to give the same number of leukocytes per unit of volume in both specimens. Then, with these suspensions, mixtures were made up exactly as before, using (1) normal human serum and normal leukocytes, (2) normal serum and patients' leukocytes, (3) patients' serum and patients' leukocytes, and (4) patients' serum and normal leukocytes. Plates were then made from these mixtures as before.

This method of study differs only in minor details from that previously used by Rosenow[1] and Tunnicliff.[2] Unfortunately a few attempts only were made to study the phagocytic activity of the leukocytes in this way. It was found difficult to run through an entire series of observations of the leukocytes in any one case, as, unless an extremely nice adjustment in the relationships between organisms and leukocytes is obtained, the results are obscured by either too many or too few organisms in the final mixtures.

The strains of pneumococcus used were two in number, both isolated shortly before from pneumonic sputum, and cultivated

[1] *Jour. Infect. Dis.*, 1910, 7, p. 429. [2] *Ibid.*, 1911, 8, p. 302.

continuously on blood agar. The one used for the earlier work was of moderate virulence (contents of one agar slant produced death by pneumococcic septicemia in a guinea-pig in one week); the organism used later was somewhat more virulent, and as tested while the work was in progress was fatal to a white mouse inside of 12 hours with a dose of one-tenth of a blood agar slant.

The results follow, the figures in the tables indicating the number of colonies on the plates:

Case 1.—J. D., male, age 33. Typical right lower lobar pneumonia; delirium tremens. Seen first on eighth day. Crisis on ninth day. Uneventful recovery.

A. ACTION OF PNEUMONIC SERUM.

	At Once	1 Hour	2 Hours	4 Hours
Equal quantities of normal serum, normal leukocytes, and pneumococci	84	31	46	164
Equal quantities of patient's serum, 8th day, normal leukocytes, and pneumococci	86	21	5	4
Equal quantities of patient's serum, 9th day, normal leukocytes, and pneumococci	71	21	7	3
Equal quantities of patient's serum, 10th day, normal leukocytes, and pneumococci	84	26	7	1
Equal quantities of patient's serum, 15th day, normal leukocytes, and pneumococci	75	19	6	2

B. ACTION OF PNEUMONIC LEUKOCYTES.

	9TH DAY		15TH DAY	
	At Once	4 Hours	At Once	4 Hours
Equal parts of normal serum, standardized normal leukocytes, and pneumococci	450	8,100	810	20,000
Equal parts of normal serum, standardized pneumonic leukocytes, and pneumococci	500	4,700	790	4,700
Equal parts of patient's serum, standardized pneumonic leukocytes, and pneumococci	510	2,300	850	2,700
Equal parts of patient's serum, standardized normal leukocytes, and pneumococci	450	2,700	800	2,050

Case 2.—W. T., male, age 27. Lobar pneumonia involving entire left lung. Seen on sixth day. Crisis on ninth day. Persisting consolidation of upper lobe at time of discharge, 11 days later.

ACTION OF PATIENT'S LEUKOCYTES.

	6TH DAY		7TH DAY		10TH DAY	
	At Once	4 Hours	At Once	4 Hours	At Once	4 Hours
Equal parts normal serum, standardized normal leukocytes, and pneumococci	48	106	730	4,200	21	2,500
Equal parts normal serum, standardized patient's leukocytes, and pneumococci	63	92	975	3,700	26	2,500
Equal parts patient's serum, standardized patient's leukocytes, and pneumococci	48	9	820	350	12	2,300
Equal parts patient's serum, standardized normal leukocytes, and pneumococci	56	30	1,300	250	21	2,500

Case 3.—H. M., male, age 24. Right lower lobar pneumonia. Seen on eighth day. Crisis on 10th day. Uneventful recovery. See Chart 1.

ACTION OF PATIENT'S SERUM.

	At Once	2 Hours	4 Hours
Equal parts normal serum, normal leukocytes, and pneumococci	130	890	About 9,000
Equal parts patient's serum, 8th day, normal leukocytes, and pneumococci	119	970	About 9,000
Equal parts patient's serum, 9th day, normal leukocytes, and pneumococci	153	640	About 3,000
Equal parts patient's serum, 10th day, normal leukocytes, and pneumococci	149	118	608
Equal parts patient's serum, 11th day, normal leukocytes, and pneumococci	149	69	66
Equal parts patient's serum, 12th day, normal leukocytes, and pneumococci	170	60	52
Equal parts patient's serum, 13th day, normal leukocytes, and pneumococci	147	109	58
Equal parts patient's serum, 14th day, normal leukocytes, and pneumococci	181	43	23

Case 4.—P. L., male, age 37. Struck on chest by car four days before admission. On entrance a limited area of dulness, with bronchophony and crepitant rales, was found anteriorly over right lower lobe. Following this, entire right lung became consolidated. Apparent critical fall of temperature on seventh day, but with no apparent improvement otherwise. Death on 11th day. Autopsy showed resolving pneumonia of entire right lung, upper lobe less advanced than others. Large white kidney.

ACTION OF PATIENT'S SERUM.

	At Once	4 Hours
Equal parts normal serum, normal leukocytes, and pneumococci	104	780
Equal parts patient's serum, 7th day, normal leukocytes, and pneumococci	137	1,310
Equal parts patient's serum, 8th day, normal leukocytes, and pneumococci	111	1,300
Equal parts patient's serum, 9th day, normal leukocytes, and pneumococci	99	1,870
Equal parts patient's serum, 10th day, normal leukocytes, and pneumococci	106	1,620
Equal parts patient's serum, 11th day, normal leukocytes, and pneumococci	124	720

One series of observations on this patient's leukocytes was also made; it failed to reveal any considerable difference from the normal in phagocytic activity.

Case 5.—C. L., male, age 58. Developed pneumonia while in hospital suffering from gastroenteritis. Seen on second day. Recovery by lysis.

ACTION OF PATIENT'S SERUM.

	At Once	4 Hours
Equal parts normal serum, normal leukocytes, and pneumococci	51	93
Equal parts patient's serum, 2d day, normal leukocytes, and pneumococci	47	141
Equal parts patient's serum, 3d day, normal leukocytes, and pneumococci	61	289
Equal parts patient's serum, 4th day, normal leukocytes, and pneumococci	64	152
Equal parts patient's serum, 9th day, normal leukocytes, and pneumococci	53	40

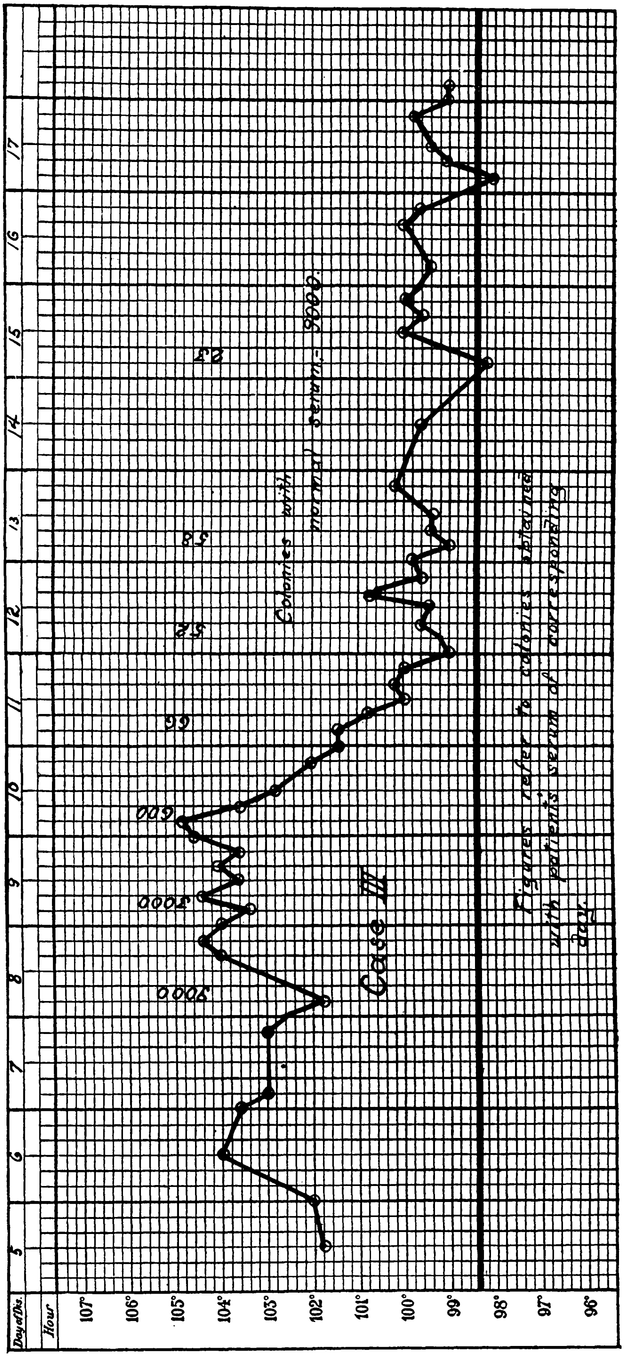
Day of Dis.
Hour
107° 106° 105° 104° 103° 102° 101° 100° 99° 98° 97° 96°
Case III
Colonies with normal serum - 9000
2000
1000
600
66
52
83
23
Figures refer to colonies obtained with patients serum of corresponding day.

CHART I.

Case 6.—W. W., male, age 31. Left lower lobe pneumonia. Seen on fourth day. Crisis on eighth day. Uneventful recovery.

ACTION OF PATIENT'S SERUM.

	At Once	4 Hours
Equal parts patient's serum, 4th day, normal leukocytes, and pneumococci	44	664
Equal parts patient's serum, 5th day, normal leukocytes, and pneumococci	39	522
Equal parts patient's serum, 6th day, normal leukocytes, and pneumococci	48	525
Equal parts patient's serum, 8th day, normal leukocytes, and pneumococci	40	112
Equal parts patient's serum, 11th day, normal leukocytes, and pneumococci	48	380

Case 7.—Mrs. S., age 53. Left lower lobe pneumonia. Seen on second day. Crisis fifth day. Uneventful recovery.

A. ACTION OF PATIENT'S SERUM.

	At Once	4 Hours
Equal parts normal serum, normal leukocytes, and pneumococci	30	630
Equal parts patient's serum, 2d day, normal leukocytes, and pneumococci	38	382
Equal parts patient's serum, 3d day, normal leukocytes, and pneumococci	30	332
Equal parts patient's serum, 4th day, normal leukocytes, and pneumococci	40	590
Equal parts patient's serum, 5th day, normal leukocytes, and pneumococci	57	155
Equal parts patient's serum, 6th day, normal leukocytes, and pneumococci	35	91
Equal parts patient's serum, 7th day, normal leukocytes, and pneumococci	31	332
Equal parts patient's serum, 9th day, normal leukocytes, and pneumococci	38	375
Equal parts patient's serum, 11th day, normal leukocytes, and pneumococci	33	500

B. ACTION OF PATIENT'S LEUKOCYTES.

	At Once	4 Hours
Equal parts normal serum, standardized leukocytes, and pneumococci	930	1,690
Equal parts normal serum, standardized patient's leukocytes, and pneumococci	990	2,150
Equal parts patient's serum, 4th day, standardized patient's leukocytes, and pneumococci	1,070	1,890
Equal parts patient's serum, 4th day, standardized normal leukocytes, and pneumococci	880	1,400

Case 8.—F. K., male, age 38. Bilateral lower lobar pneumonia. Seen on third day. Death on eighth day, without crisis.

A. ACTION OF PATIENT'S SERUM.

	At Once	4 Hours
Equal parts normal serum, normal leukocytes, and pneumococci	490	845
Equal parts patient's serum, 3d day, normal leukocytes, and pneumococci	382	500
Equal parts patient's serum, 4th day, normal leukocytes, and pneumococci	550	435
Equal parts patient's serum, 5th day, normal leukocytes, and pneumococci	430	551
Equal parts patient's serum, 6th day, normal leukocytes, and pneumococci	420	465
Equal parts patient's serum, 7th day, normal leukocytes, and pneumococci	435	580
Equal parts patient's serum, 8th day, normal leukocytes, and pneumococci	360	980

B. ACTION OF PATIENT'S LEUKOCYTES.

	3D DAY		4TH DAY	
	At Once	4 Hours	At Once	4 Hours
Equal parts normal serum, standardized normal leukocytes, and pneumococci	2,960	625	540	99
Equal parts normal serum, standardized patient's leukocytes, and pneumococci	3,190	845	550	2,600
Equal parts patient's serum, standardized patient's leukocytes, and pneumococci	2,900	435	430	330
Equal parts patient's serum, standardized normal leukocytes, and pneumococci	2,900	380	530	22

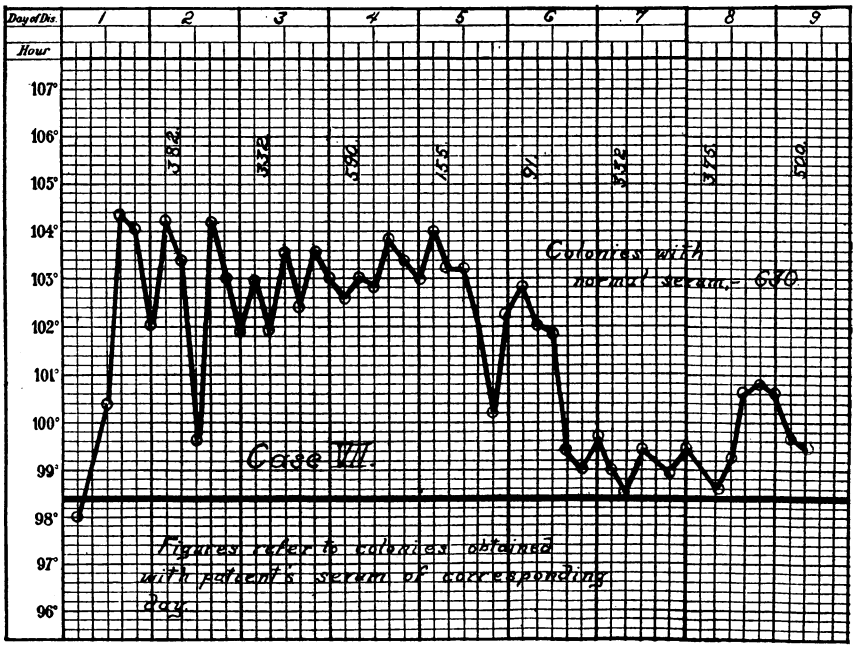

CHART 2.

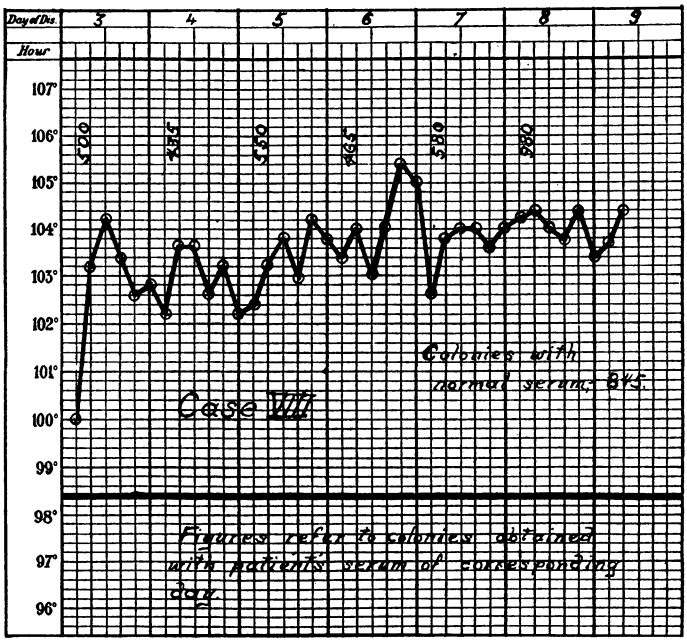

CHART 3.

From the foregoing results it would appear that in lobar pneumonia, as a rule, there is a development of antipneumococcal bodies usually progressing up to the crisis and reaching its maximum at or a little after this time. In two cases (4 and 5) this was absent, and in one (8) it was not well marked. But Case 4, although there was a crisis, if temperature alone be taken as an indication of this, showed no improvement at this time, and died not long after; so that the crisis at least was not typical. Case 5 recovered by lysis, and it is perhaps significant that late in the disease the serum in this case as well showed evidence of increased antipneumococcal power. Case 8, again, was one of exceptional severity; while at first there was evidence of antibody formation, this never became very marked, and disappeared entirely the day before death. Case 7 is peculiar in that there was little or no evidence of increase in antibodies until just before the crisis, which came on the fifth day, and that this increase was extremely transitory. The same transitory character of the increase in destructive power is shown also in Case 2, while in others again—1, 3, and 6—it persisted as long as the patient was kept under observation.

As to the nature of the particular antibodies concerned, the incomplete character of the experiments is not such as to elucidate this completely. Both agglutinins and opsonins have previously been demonstrated in pneumonic serum, and either of these might give results of the sort obtained and, especially, the opsonins. That these bodies, at least in part, are opsonins is indicated by the fact that the number of plate colonies was in some degree dependent on phagocytosis, as is shown by the dissimilar results frequently obtained when both normal and pneumonic leukocytes were used. An experiment, the result of which points in the same direction, is as follows:

	At Once	4 Hours
Normal serum, normal leukocytes, and pneumococci	450	6,000
Normal serum, salt solution, and pneumococci	490	260
Case 3, serum, salt solution, and pneumococci	480	92
Case 3, serum, normal leukocytes, and pneumococci	450	317

The great reduction in colonies in these mixtures in which serum alone was used is probably to be explained by the fact that the lack

of hemoglobin in them made them unfavorable media for the growth of pneumococci. But it will be observed that the relative disproportion between these mixtures in which leukocytes are present is much greater than in those in which serum alone was used. However, the fact that the colonies in the mixture with pneumonic serum alone were decidedly fewer than in that containing normal serum would indicate the presence as well of bodies not concerned in phagocytosis—presumably agglutinins.

In regard to the behavior of the leukocytes, unlike Rosenow,[1] who found in all of his cases heightened leukocytic activity, and in agreement with Tunnicliff,[2] who obtained instances both of increased and diminished phagocytic activity on the part of the leukocytes in pneumonia, the few cases studied here from this viewpoint showed examples of both modifications. Cases 1 and 2 gave evidence of greater phagocytic activity of the leukocytes than normal, and Cases 7 and 8 of the reverse. In these cases, it would be impossible to assert that the severity of the attack had any relation to this behavior of the leukocytes as was observed by Tunnicliff in her cases.

SUMMARY.

1. In most cases of lobar pneumonia there is obtained by the plate method distinct evidence of increased antipneumococcal power, as a rule, greatest at or just after the onset of crisis, and lasting for variable periods afterward.

2. The antibodies are apparently in part at least opsonins, and their activity can be manifested in proper circumstances even with organisms of some degree of virulence.

3. The cases in which this apparently characteristic increase of antipneumococcal power did not occur presented irregularities either in course or termination.

[1] *Jour. Infect. Dis.*, 1906, 3, p. 683. [2] *Ibid.*, 1911, 8, p. 302.